# THE EFFECT OF ENVIRONMENTAL ENRICHMENT ON THE WITHDRAWAL OF OPIOID DEPENDENT RATS

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by

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# THE EFFECT OF ENVIRONMENTAL ENRICHMENT ON THE WITHDRAWAL OF OPIOID DEPENDEDNT RATS

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## ABSTRACT

Opioid use disorder (OUD) is understood to be a chronic relapsing disorder that, even though successful recovery is possible with appropriate treatment, engages and effects the function in brain reward pathways in the central nervous system (CNS), potentially leading to higher rates of relapse (Blanco & Volkow, 2020). Continual consumption of opioids can potentially lead to the development of dependence and/or tolerance of the drug. Patients that develop a dependence on the drug often have a difficult time weaning off it due to the discomfort of withdrawal. The current study assessed if an enriched environment (EE) mediated the withdrawal symptoms within an opioid dependent rat. Previous research has demonstrated the beneficial effects EE has on withdrawal and drug seeking behavior (Glaj, Barrera, & Ranaldi, 2019). If EE does mediate withdrawals, this can provide better treatment interventions for those seeking help.

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# INTRODUCTION

The growing misuse of opioids is considered the most profound public health issue our nation has faced (Vadivelu, Kai, Kodumudi, Sramcik, & Kaye, 2018). In 2017, more than 70,000 Americans died from a drug overdose, the majority of them originating from opioid or opioid-based prescription drugs (Galaj, Barrera, & Ranaldi, 2019). Opioids, such as oxycodone (Oxycontin) are commonly prescribed for pain management (Zanni, DeSalle, Deutsch, Barr, & Eisch, 2020) but the increasing easy access to these medications is a contributing factor for higher rates of addiction, tolerance, and dependence around the world (Weiss & Rao, 2016).

Opioids have been and are prescribed for the treatment of severe acute, surgical, and cancer pain (Furlan, Sandoval, Mailis-Gagnon, & Tunks, 2006). Distinction should be made when prescribing opioids to treat cancer pain and acute, chronic non-cancer pain (CNCP) as their significance and management vary (Shipton, Shipton, & Shipton, 2018). For instance, prescribing opioids for the treatment of CNCP includes the possibility of turning into a destructive phenomenon leading to an overwhelming impact psychologically, socially, physically, and economically (Shipton et al., 2018). Over prescription of opioids to CNCP patients may also be a contributing factor to widespread misuse of opioids and illicit opioid overdose-related deaths (Shipton et al., 2018).

Conceptualizing opioid use disorder and the many contributing factors associated with its development and maintenance may help clinicians better comprehend and provide the best course of treatment and how to achieve a reduction in relapse rates and maintain long-term abstinence (Blanco & Volkow, 2019).

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**Dependence and Tolerance**

Some of the factors that help define opioid addiction include the compulsion to seek out and take opioids and a lack of control in limiting opioid intake (Strang, Volkow, Degenhardt, Hickman, Johnson, Koob, Marshall, Tyndall, & Walsh, 2020). The development of tolerance and physical dependence are effects produced from the consumption of opioids in cancer and noncancer pain populations, but they should not be confused with addiction (Jage, 2004). Tolerance is developed when there is desensitization of the opioid receptors, often leading to consuming a higher dosage of the drug to get the same effect (Wang, Chen, Lee, & Cheng, 2019). Dependence arises from the repeated use of opioids combined with craving the drug in order to prevent and/or mediate withdrawal symptoms (Blanco & Volkow, 2019; Wang, 2018). Risk of relapse is heightened due to the increase in tolerance of opioid receptors and the neurobiological changes that take place within the central nervous system (CNS) caused by repeated misuse and abuse of opioids (Wang et al., 2019).

From a clinical standpoint, opioid withdrawal is a major contributing factor in the development of opioid dependence and addictive behaviors (Kosten & George, 2002). When an individual uses opiates (oxycodone, morphine, hydrocodone, etc.), the compound then binds to mu opioid receptors (MORs) triggering the biochemical brain processes relating to reward and pleasurable feelings (euphoria) (Kosten & George, 2002). This activates the mesolimbic system in the brain that then generates signals to the ventral tegmental area (VTA) to release the neurotransmitter (NT) dopamine (DA) into the nucleus accumbens (NAc) (Kosten & George, 2002). The release of DA is also linked to feelings of pleasure (Kosten & George, 2002). With this, the brain then creates memories that can be associated with the pleasurable feelings and the environment in which they occur, often leading to drug-

seeking related behaviors (Kosten & George, 2002). Understanding all the factors, systems, and brain regions that play into the development of tolerance, dependence, and ultimately addiction is crucial for effective interventions to prevent and/or stop cognitive deterioration (Wang et al., 2019).

**Withdrawal**

As opioid use disorder (OUD) prevalence continues to rise, opioid withdrawal will continue to rise as well. Opioid withdrawal is a condition resulting from the reduction or cessation of opioid use in an individual that developed a dependence following chronic usage of the drug (Stark, 2020). Current medications used for the treatment of OUDs include methadone (a full mu-opioid receptor agonist that reduces illicit opioid use gradually), buprenorphine (partial mu-opioid receptor agonist), and naltrexone and naloxone (mu-opioid antagonists used to reverse overdoses) (Blanco & Volkow, 2019; Wang, 2018). There are three major opioid receptors found throughout the (CNS): mu, kappa, and delta (Pergolizzi, Raffa, & Rosenblatt, 2020). They are part of the body's endogenous opioid system, which is responsible for the regulation of pain, reward, and addictive behaviors (Contet, Kieffer, & Befort, 2004). When endogenous (endorphins, enkephalins, and dynorphins) and exogenous opioids (opioids that are introduced from outside the body) bind to opioid receptors, mu specifically, this interaction triggers a cascade of chemical signals in the CNS such as reducing the excitability of neurons leading to pain relief and euphoria (Contet et al., 2004). This interaction additionally increases the production of the neurotransmitter DA, which is responsible for reward-seeking behavior, attention, and mood (Contet et al., 2004).

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria, signs and symptoms of opioid withdrawal in humans include lacrimation (flow of

tears) or rhinorrhea (excess nasal drainage), nausea/vomiting, diarrhea, pupillary dilation and photophobia (light sensitivity), insomnia, yawning, and autonomic hyperactivity (hypertension, hyperthermia, tachycardia, sweating, hyperreflexia, tachypnea) (Shah & Huecker, 2021). In non-human animals, spontaneous and precipitated withdrawal behaviors include head movement (lateral and rotary movement of the head), high walking (elevated torso or spine curved upwards), jumping, ptosis (one or both eyelids closed or semi closed), wet dog shakes (rapid shaking of whole body), burrowing, diarrhea, yawning, stretching, and teeth chattering (constant chattering of teeth/movement of the jaw) (Zanni et al., 2020).

Severity of withdrawal is dependent upon dosage, duration, and continuity of opiate usage (Shah& Huecker, 2021). It can also be precipitated by administration of opioid agonists (buprenorphine, naloxone, and naltrexone) (Shah & Huecker, 2021). Opioid withdrawal causes varying levels or discomfort and distress; therefore, it is important to recognize these withdrawal symptoms in order to facilitate symptom relief for patients and establish proper treatment methods for those individuals seeking help (Shah & Huecker, 2021).

**Environmental Enrichment**

Although pharmacotherapies such as buprenorphine, methadone, and naltrexone are often some of the first steps taken against opioid use disorders, there have been other implementations like behavioral interventions (e.g., cue-exposure therapy and contingency management therapy), but they have also demonstrated to have short lasting effectiveness (Galaj, 2019). Environmental enrichment (EE) is the process of exposing laboratory animals to a social and/or physical type of stimulation (Simpson & Kelly, 2011). Physical enrichment consists of the addition of structures that allow exercise like running wheels, plastic tunnels,

gnawing toys and other appropriately sized toys not typically incorporated into standard environments (SE) of living. Social enrichment or social interaction is simply housing more animals together, if and when possible.

Previous research has demonstrated that rats housed in larger cages filled with objects used for physical enrichment (novel objects or toys and running wheels), had larger cerebral cortices and greater amounts of cortical acetylcholinesterase (AChE) when compared to rats in isolation and/or standard environments of living (Galaj et al., 2019). In addition, EE has demonstrated the modification of neurochemical parameters of brain-derived neurotrophic factor (BDNF, a protein that helps the regulation of synaptic plasticity) and cholinergic and glutamatergic systems, all which are important for learning and memory (Galaj et al., 2019). In this same study, EE induced neural adaptations in the nucleus accumbens and dorsal striatum, the regions responsible for motivation and habit formation, respectively (Galaj et al., 2019). Another study conducted by Imperio and colleagues (2018) found that EE decreased the willingness to work for heroin and reduced the addiction-like behavior in heroin experienced rats.

Researchers suggest that since EE is a behavioral intervention already applied to preclinical and clinical populations, it provides the understanding for significant changes regarding neuronal and behavioral functions (Galaj et al., 2019). Overall, previous studies indicate that the implementation of EE may decrease drug-seeking behavior and diminish cue-induced relapse-like behaviors (Imperio, McFalls, Hadad, Blanco-Berdugo, Masser, Colechio, Coffey, Bixler, Stanford, Vrana, Grigson, & Freeman, 2018).

Given the benefits noted and the lack of effective treatments for the prevention of relapse, EE produces protective and therapeutic effects against drug addiction in both human

and non-human animal studies and can be a contribution to the development of better therapy methods that will help attenuate high relapse rates, maintain long-term recovery, and may help individuals struggling with substance use disorders regain control over their impulsivity (Galaj et al., 2019).

**Treatment**

The opioid epidemic has and will remain a crucial issue that will not resolve easily. Opioid use disorders and opioid addiction is a chronic mental illness that may cause an opioid dependent individual to experience multiple relapses and remissions throughout their lifetime (Wang, 2019). This may be due to changes in the regions of the brain involved in reward processing and reward-related learning, incentive motivation, and habit forming and compulsivity, all behaviors that relate to drug addiction development (Galaj et al., 2019).

Previously mentioned, there are currently three medications approved for treating OUDs: buprenorphine, methadone, and naltrexone (extended release) (Volkow & Collins, 2017). These medications have proven to be effective in improving mortality rates, treatment retention, and remission, but many individuals remain untreated (Wakeman, Larochelle, Ameli, Chaisson, McPheeters, Crown, Azocar, & Sanghavi, 2020). Even so, it is estimated that 5.3 to 15.3% of individuals maintain abstinence from drugs for more than a year after the cessation of pharmacotherapy, leading to staggering rates of relapse (Galaj et al., 2019). Previous studies indicate that these medications paired with psychosocial support are becoming the standard care for reducing illicit opioid use, overdoses, relapse rate, and can improve social function (Volkow & Collins, 2017) but there is still an urgent need for more effective treatment methods.

As literature continues to expand, intervention strategies involving EE have proven to be highly effective in the treatment of psychological and neurological disorders like addiction (Galaj et al., 2019). In the human population, there have been concerns about the translation of therapeutic benefits of EE to those individuals struggling with substance use disorders (SUDs). The suggestion of incorporating enhanced cognitive stimulation, physical activity, and social interaction into the everyday lives of drug-addicted individuals can hinder drug-related behaviors (Galaj et al., 2019). In addition, the strengthening of social support from non-drug using family members, friends, colleagues, program groups, sponsors, and peers play a crucial role in the recovery process of these individuals and has shown longer participation of treatment programs and prolonged abstinence from drug usage (Galaj et al., 2019).

**Hypothesis/Purpose**

The aim of this study was to investigate whether exposure to an enriched environment during the induction of opioid dependence mediates withdrawal symptoms in opioid-dependent female rats. We hypothesized that the oxycodone enriched environment (OXY/EE) group would show fewer signs of withdrawal when compared to the oxycodone standard environment (OXY/SE) group.

# METHODS

## Animals and Housing

Thirty-two female Sprague-Dawley rats (aged one month) were ordered from ENVIGO and transported to the Jackson Street Psychology Laboratory. Upon arrival, the rats were housed two to a cage in a semi-self-contained housing system (Optirat). They had *ad libitum* access to food and water and the vivarium maintained a 12-h light/dark cycle for the entirety of the study. Due to some unforeseen circumstances, the rats acclimated to their environment for approximately one month after arrival. EE

## Standard and Enriched Environments

All 32 rats were divided, at random, into four groups ($n$=8): saline standard environment (Sal/SE), saline enriched environment (Sal/EE), oxycodone standard environment (Oxy/SE), and oxycodone enriched environment (Oxy/EE). Standard environment of living consisted of generalized cages measuring 14 in. L x 19.1 in. W x 8.6 in. H. Enriched environment of living consisted of the same cage with the inclusion of a chewing toy and longer handling/interaction time. In addition, EE rats were placed in environmental cages measuring 18 in. L x 14 in. W x 23 ¼ in. H for an hour everyday. These cages also included chewing toys and running wheels.

## Drug Administration

After the animals were randomly assigned to their experimental/control group and the first week of habituation was completed, they underwent a two-week (14 consecutive days) period of oxycodone and saline administration (subcutaneous injections once daily) in

order to build up their dependency on the drug. Dosages administered are consistent with extant literature (10.0 mg/kg) (Miladi-Gorji, Rashidy-Pour, & Fathollahi, 2011).

**Procedure**

Week one of the current study was considered a habituation week, where all 32 of the subjects were handled for 5 minutes each for 7 consecutive days. Following this, all animals in the environmental enrichment group were placed in the designated EE cages for an hour after handling. There were two cages, Sal/EE was in one ($n$=8) and Oxy/EE was in the other ($n$=8). Upon the completion of habituation, each rat received subcutaneous injections of either oxycodone or saline (dependent on the group they were randomly assigned to) for the following 14 consecutive days. Rats in the standard environment of living were returned to their home cages after injections. Rats in the enriched environment of living were placed in the designated EE cages for an hour and then returned to their home cages.

On day twenty-one (after 14 consecutive days of drug/no-drug administration), the rats were administered 1.0 mg/kg of naloxone (Zanni et al., 2020) and withdrawal behaviors were then assessed. Each group was administered the 1.0 mg/kg of naloxone at a time and withdrawal behaviors were then recorded for 30 minutes.

Observers blind to the study were asked to analyze the 30-minute videos taken on withdrawal day. Interrater reliability was then calculated from the scores collected from the raters. Withdrawal symptoms were rated according to adaptations from previous studies that used the Gellert-Holtzman withdrawal rating scale (Harvey-Lewis, Perdrizet, & Franklin, 2012; Miladi-Gorji et al., 2011; Zanni et al., 2020): teeth chattering, diarrhea (0 = absent; 1 = mild; 2 = moderate; 3 = marked), ptosis (0 = absent; 1 = mild; 2 = moderate; 3 = marked)

(Zanni et al., 2020), and wet dog shakes (1-2 shakes = 2; 3-4 = 3; 4 or more = 4) (Miladi-Gorji et al., 2011). After all groups went through withdrawal, they were returned to their home cages. Upon completion of data collection, all 32 rats were euthanized.

## RESULTS

Interrater reliability was calculated with Cronbach's Alpha, a measurement used for internal consistency (reliability), from the summated scores collected from each rater after observing opioid withdrawal (see Table 1).

| **Cronbach's Alpha** |
|---|
| 0.345 |

**Table 1**: Interrater reliability for all withdrawal behaviors.

The scale had a low level of internal consistency, as determined by a Cronbach's alpha of 0.345. These results indicate a low level of internal consistency for the summation of withdrawal behaviors observed between raters.

Furthermore, since two of the four withdrawal symptoms (diarrhea and ptosis) were scored based on a rating scale, all variables were converted into Z-scores to combine all withdrawal behaviors to be able to compute for an overall withdrawal score. To be able to test the hypothesis that environmental enrichment mediates withdrawal symptoms within an opioid dependent rat.

A One-Way ANOVA was conducted to determine the effect of environmental enrichment on the withdrawal symptoms in opioid dependent rats. There were no statistical differences between environmental enrichment and the anticipated withdrawal symptoms, $F(3, 15) = 2.37$, $p > 0.05$ ($p = 0.111$). Means and standard deviation for all groups can be seen in table 2.

| Group | N | Mean | SD |
|---|---|---|---|
| SE/Oxy | 8 | 1.65 | 2.61 |
| SE/Sal | 8 | -0.415 | 2.05 |
| EE/Oxy | 8 | -0.080 | 2.23 |
| EE/Sal | 8 | -1.152 | 1.30 |

**Table 2**: Means and standard deviations of zscores for One-Way ANOVA

A Two-Way ANOVA was conducted to compare the two independent variables (drug/no drug and environment) to overall withdrawal. Results showed a significant difference between saline and oxycodone administration when examining overall withdrawal, $F(3, 28) = 1.80$, $p = 0.169$. Estimated marginal means for the variables can be seen in Table 3.

| Drug Group | Environment | Mean | SD |
|---|---|---|---|
| Oxy | SE | 1.65 | 2.6 |
| | EE | -0.08 | 2.0 |
| Saline | SE | -0.41 | 2.2 |
| | EE | -1.15 | 1.3 |

**Table 3**: Estimated marginal means and standard deviations for Two-Way ANOVA

## DISCUSSION

Based on previous research, the current study hypothesized that an enriched environment would mediate withdrawal symptoms in an opioid dependent rat. Findings showed that enriched environments had no overall effect on withdrawal, but there was a difference between saline and oxycodone, suggesting that oxycodone had more of an impact on withdrawal when compared to the saline group, which was to be expected.

Even though EE has shown to be useful for the reduction of drug seeking behavior, modifying neural circuits in regions of the brain relating to compulsive drug seeking, and has reduced stress, which plays a role in relapse, prolonged EE intervention in pre-clinical settings should be considered for future studies (Galaj et al., 2019). Additionally, drugs of abuse such as opiates have profound neurobiological effects both structurally and functionally, even after reduction or cessation of drug use (Imperio et al., 2018). Possibility of introducing EE *after* the acquisition of opioid self-administration, heroin specifically, may facilitate abstinence for longer periods of time (Galaj et al., 2019).

### Limitations

One limitation during the current study was the groups sample size ($n$ = 8). With a larger sample size, there may have been the possibility of observing proper withdrawal behaviors and greater interpretation of results within the groups (Hackshaw, 2008).

Although EE rats were exposed to both physical and social enrichment, their time in the designated EE cages was restricted to only an hour per day. Significant differences may have been seen if rats were allowed to be permanently placed or housed in an enriched environment for the entirety of the current study. In addition, incorporating isolation instead

of standard environment of living might have provided a greater understanding on the severity of withdrawal in opioid dependent rats (Galaj et al., 2019).

**Implications and Concluding Remarks**

Despite the encouraging results of pharmacological treatment approaches to combat substance use disorders like OUDs, effective long-term treatments are still limited (Imperio et al., 2018). The present study examined the effect of exposure to EE following the development of dependence of opioids within rat models. Although results showed no supporting evidence for the hypothesis made, previous research has demonstrated that EE can still be useful in the treatment of SUDs in rodent models (Yazdanfar, Farnam, Sadigh-Eteghad, & Mahmoudi, 2021).

The increasing use and misuse of opioids will continue to cause public health challenges within our nation (Zanni et al., 2018) but the expansion of new approaches for treatment involving the strengthening of social support may positively impact those individuals suffering from OUDs and properly receive the care they need.

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# APPENDIX

## IACUC APPROVAL

ANGELO STATE UNIVERSITY
College of Graduate Studies & Research
*Institutional Animal Care & Use Committee*

April 21, 2021

Dr. Steven Brewer, Assistant Professor
Department of Psychology and Sociology
Angelo State University
San Angelo, TX 76909

Your proposed project titled, "The Effect of Environmental Enrichment on the Withdrawal of Methamphetamine Dependent Rats" was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective April 21, 2021 and it expires three years from this date; however, an annual review and progress report form (www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport) for this project is due on August 15 of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 2020-107. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Sincerely,

Chase Runyan, Ph.D.
Co-Chair, Institutional Animal Care and Use Committee

## BIOGRAPHY

Maria del Carmen Molinar Muniz graduated from Angelo State University with a Bachelor of Science degree in 2019. Her major was Health Science Professions with a concentration in Allied Health/Physical Therapy. She is currently a Masters student in the Experimental Psychology program at Angelo State. Maria del Carmen did not have any experience when it came to animal research or the formal presentation of research for that matter, but she now feels she has gained more knowledge and confidence in both areas. Maria del Carmen does not yet know what is in store for her after the completion of the program, but she is confident that her experience here will open doors of opportunities and is excited for what is to come.